Product Disposition in Periplasm Soluble Protein vs Aggregate

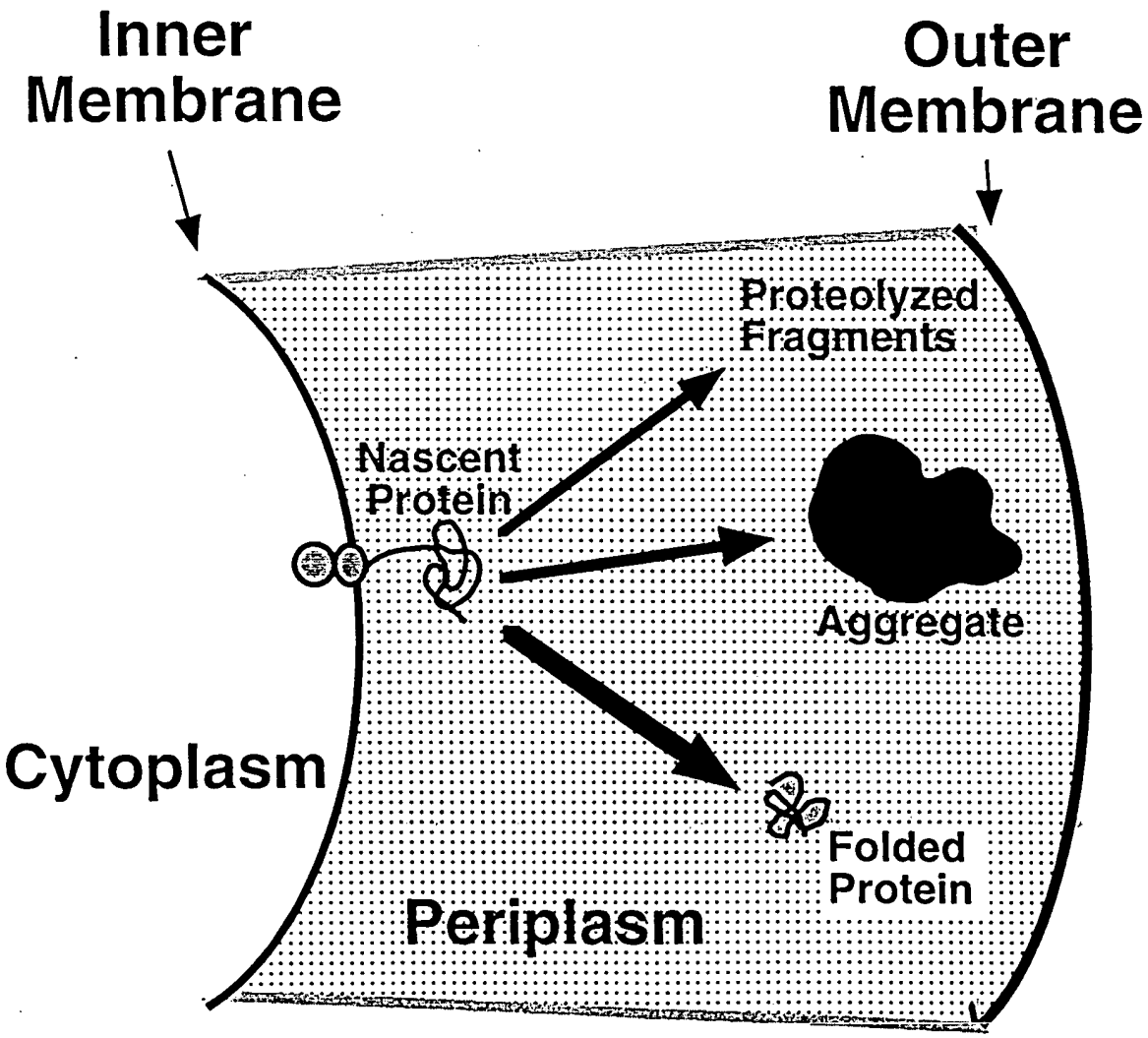


Fig. 1

Mechanical Disruption Leads to Incomplete Recovery of IGF-I Aggregates

Hart et al., Bio/Technology 12:1113 (1994)

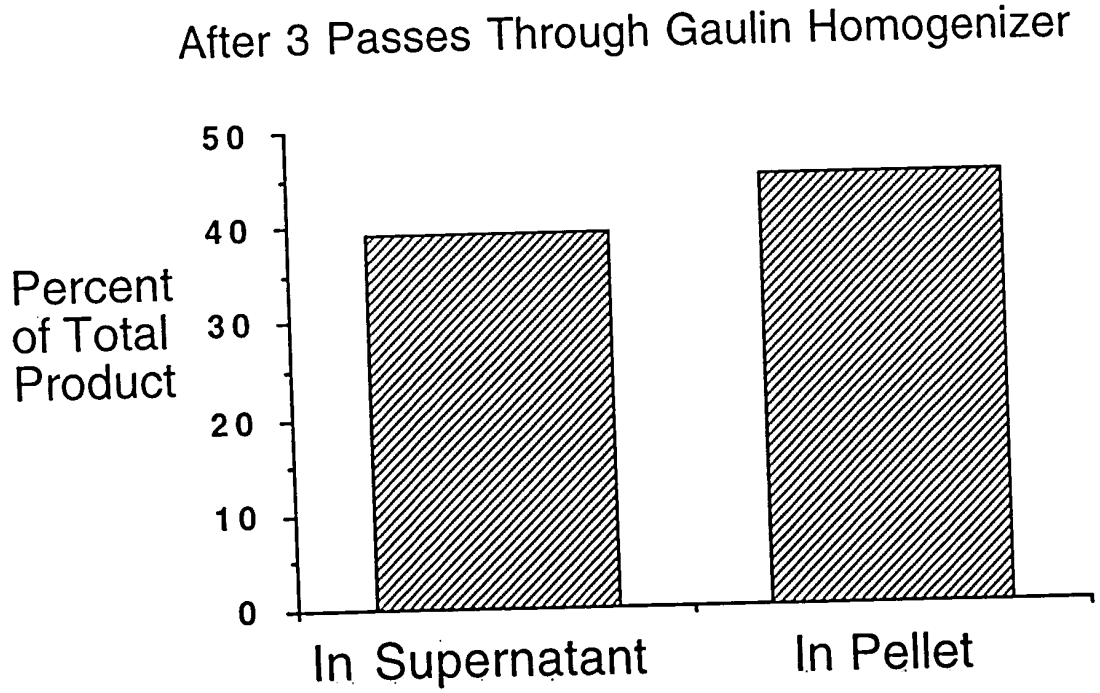


Fig. 2

pIGFLysAra Plasmid Construction

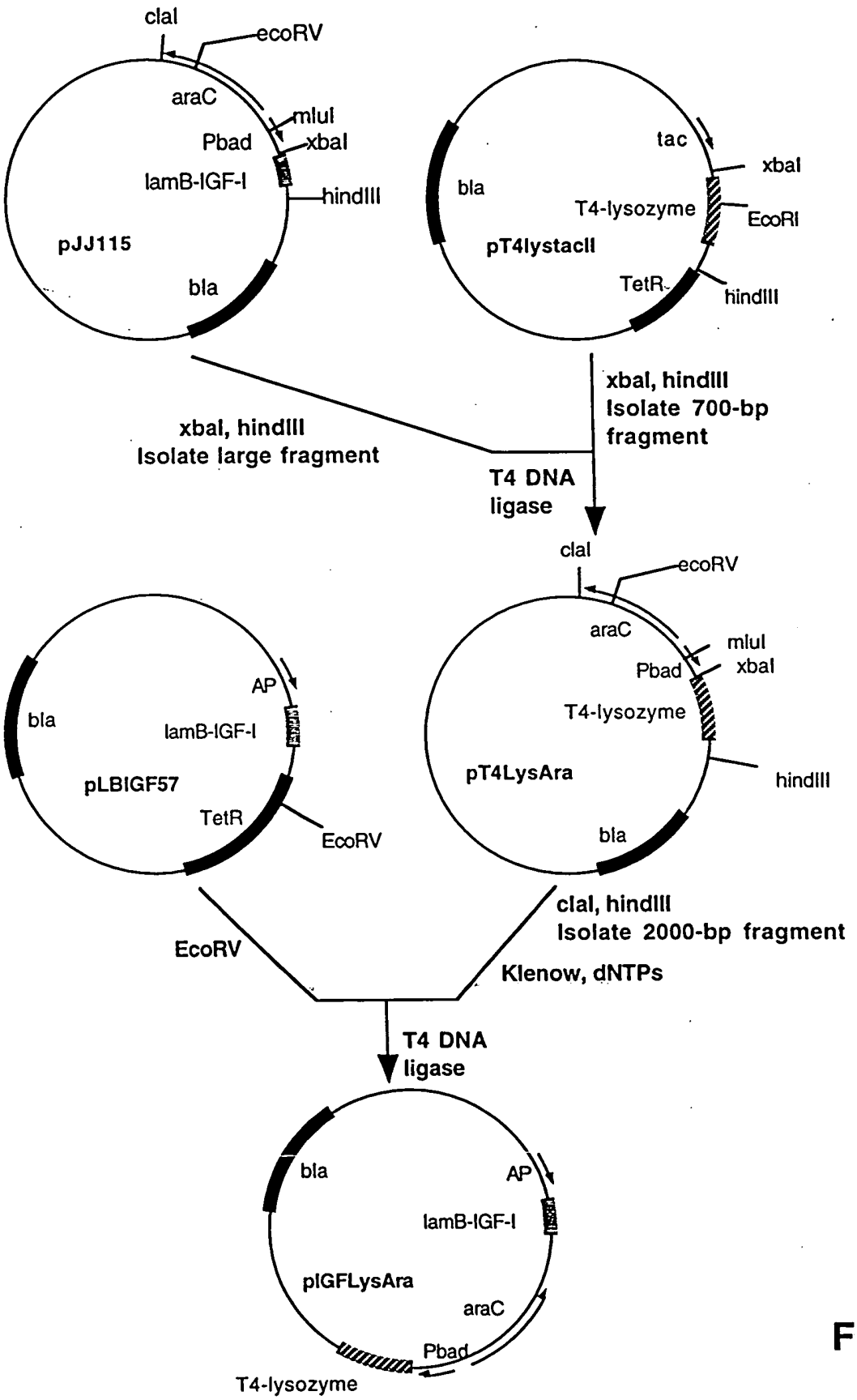


Fig. 3

Co-expression of T4-lysozyme and IGF- I by *E. coli*

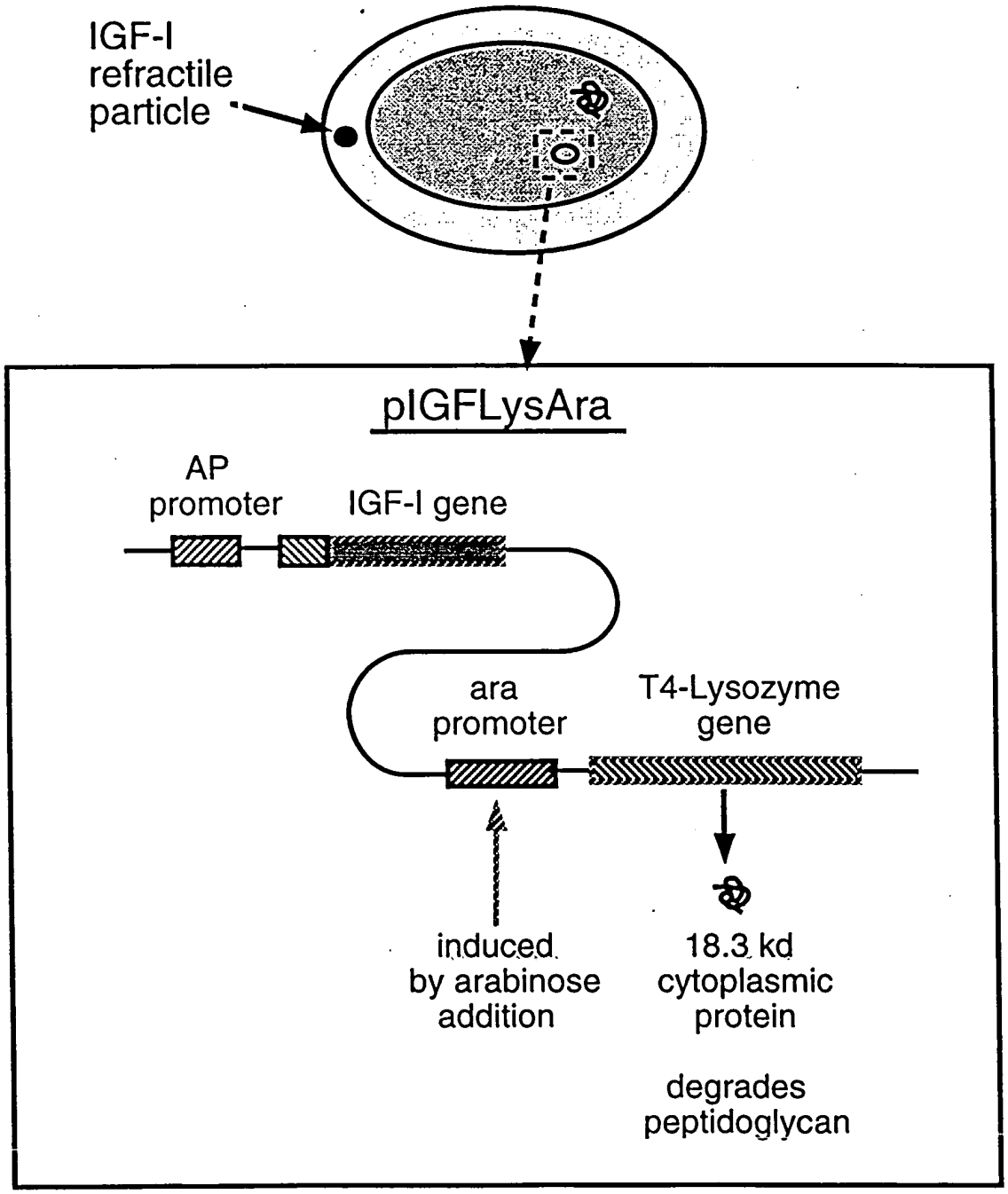


Fig. 4

rhIGF-I Fermentation Process

With Co-expression of T4-Lysozyme

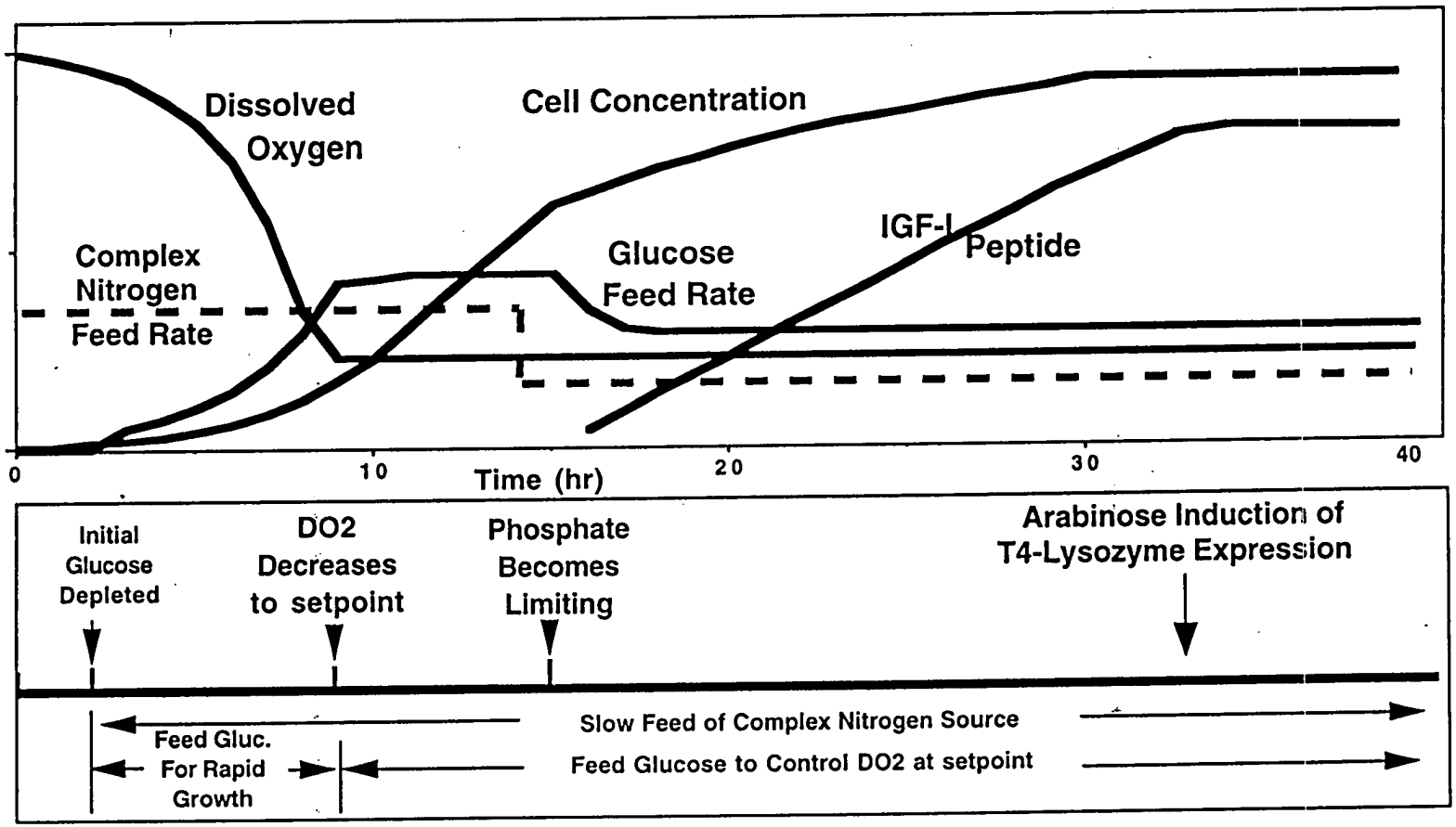
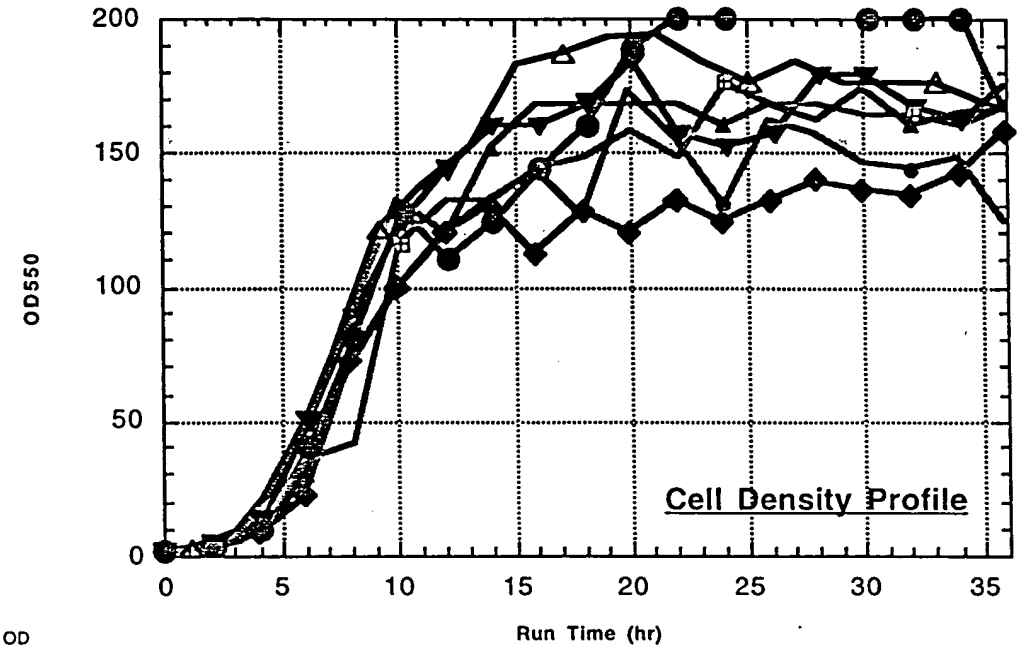


Fig. 5

Effect of Arabinose Induction for T4-lysozyme Co-expression on Cell Density Profile

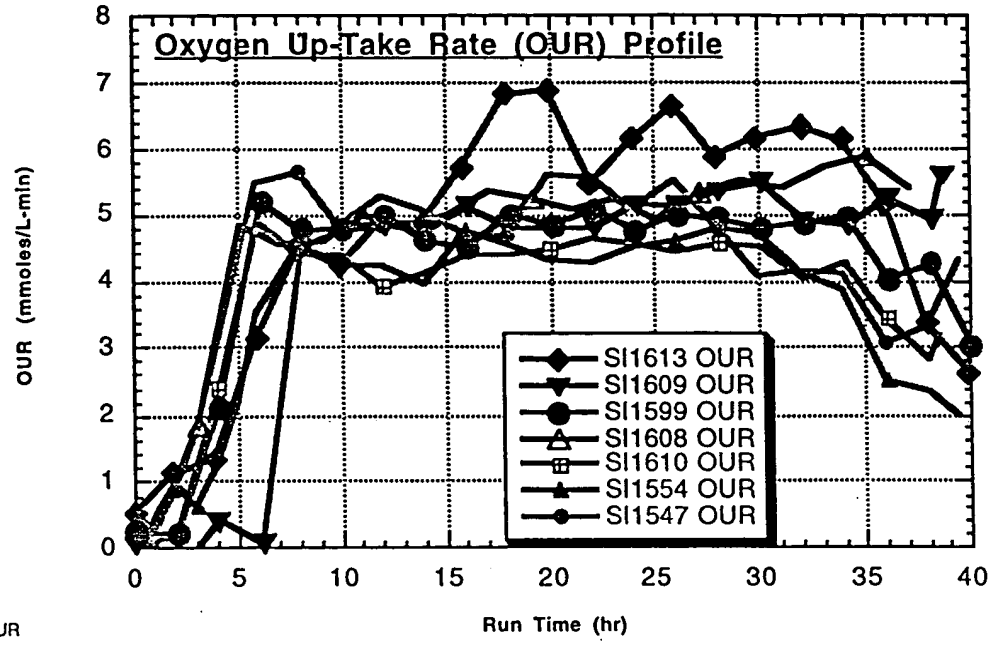


Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Fig. 6

Effect of Arabinose Induction for T4-lysozyme Co-expression on Cellular Respiration



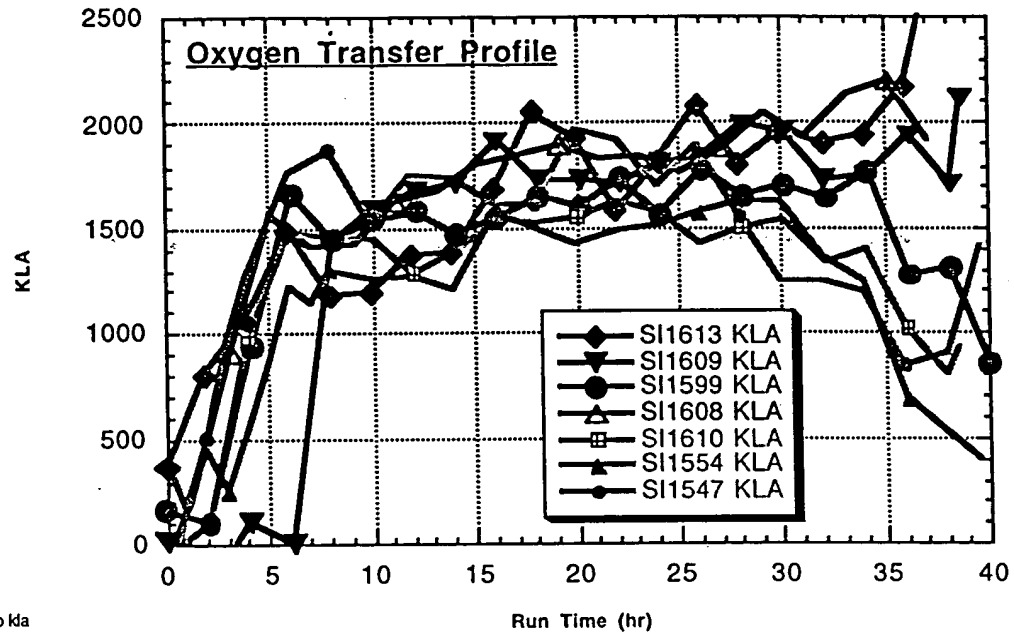
T4-lyso OUR

Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Fig. 7

Effect of Arabinose Induction for T4-lysozyme Co-expression on Oxygen Transfer during Fermentation



Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Fig.8

Effect of T4-lysozyme Co-expression on IGF-I Accumulation

Arabinose Induction of pBAD Promoter

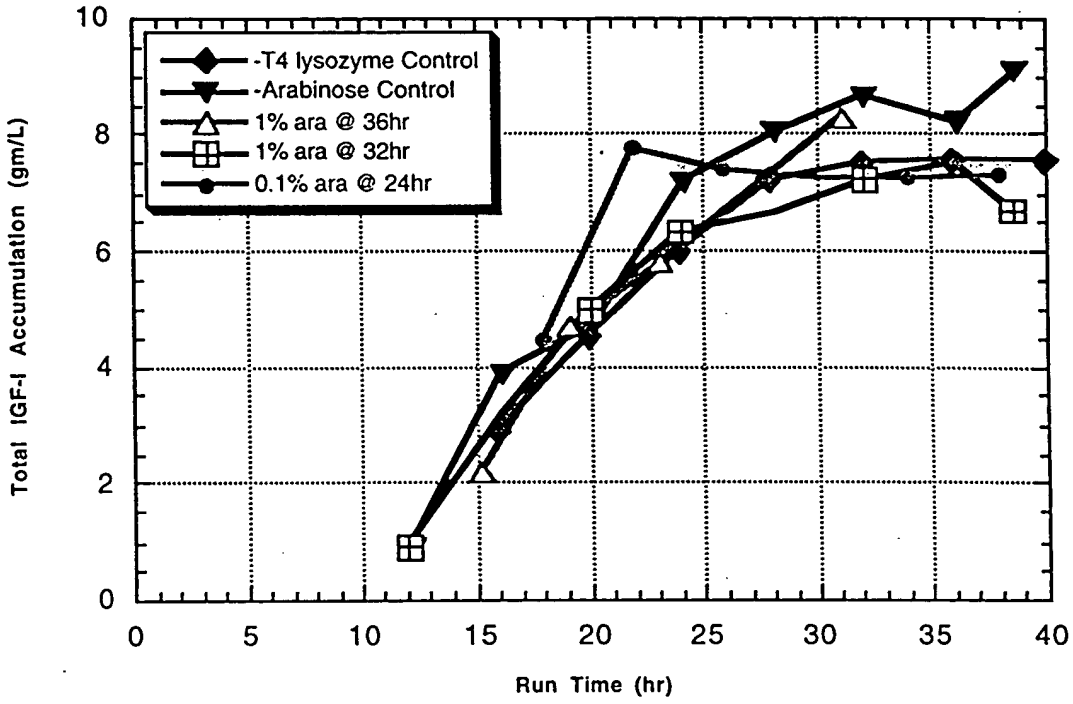


Fig. 9

Facilitating Product Isolation Procedure With T4 Lysozyme Expression

1) Induce *in vivo* T4 Lysozyme Expression

* Sequested in cytoplasmic compartment

2) Isolate IGF-I Aggregates

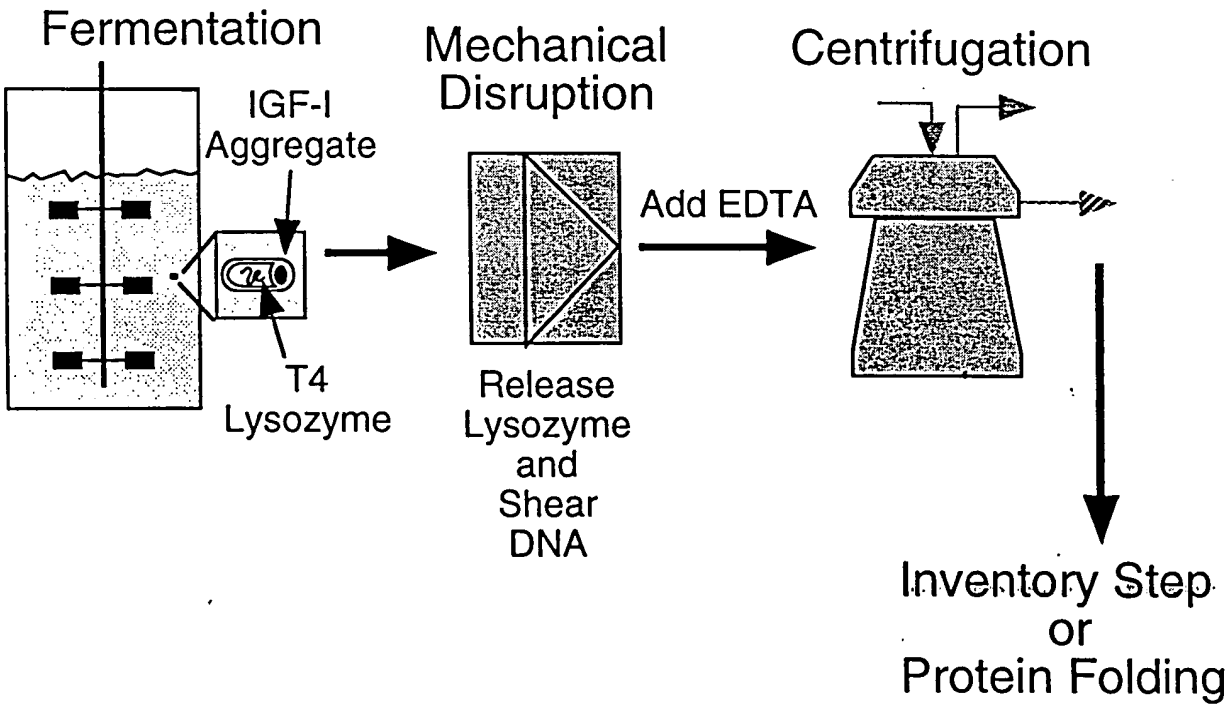
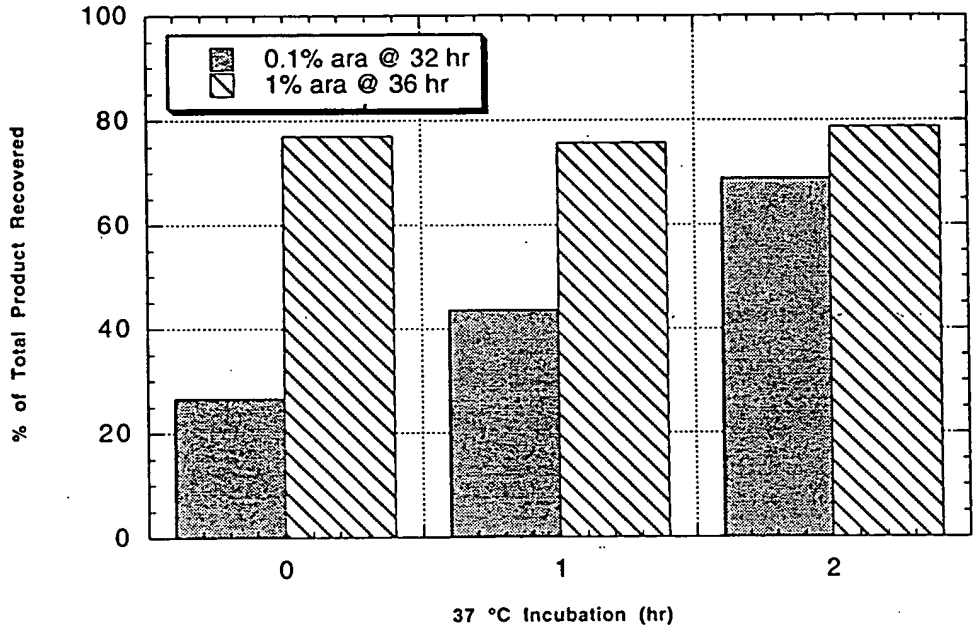


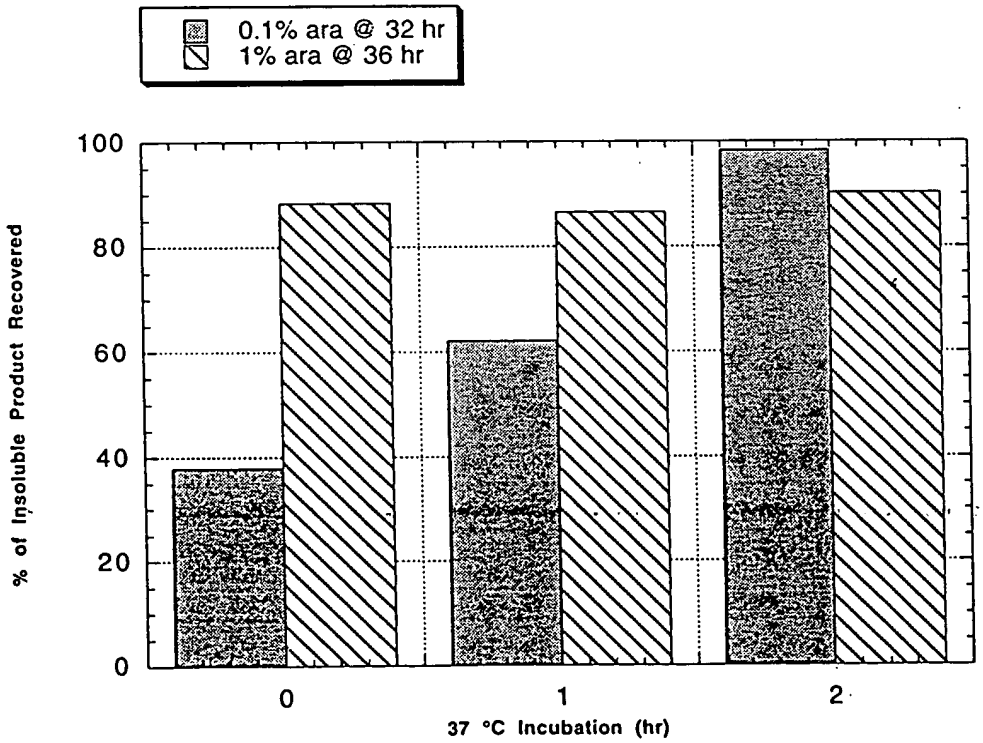
Fig. 10

FIG 11

Co-Expression of T4-Lysozyme with IGF-I for Improved RP Recovery



A



B

* RP recovered by centrifugation at 5000 rpm X 30 min in Sorval centrifugation using GSA rotor

37C incub. time vs RP yield

Facilitating Product Isolation With T4 Lysozyme Co-Expression

Results:

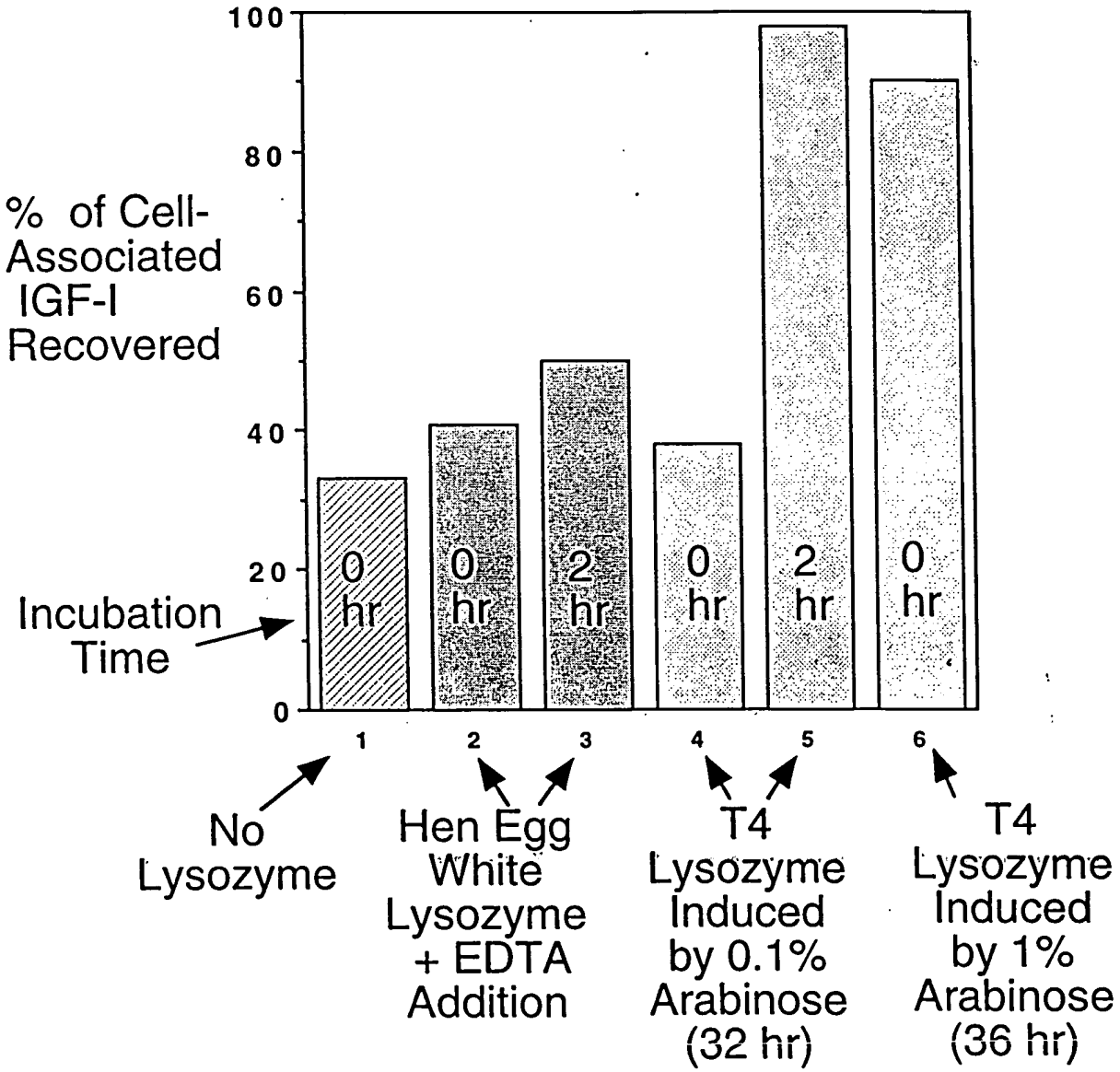
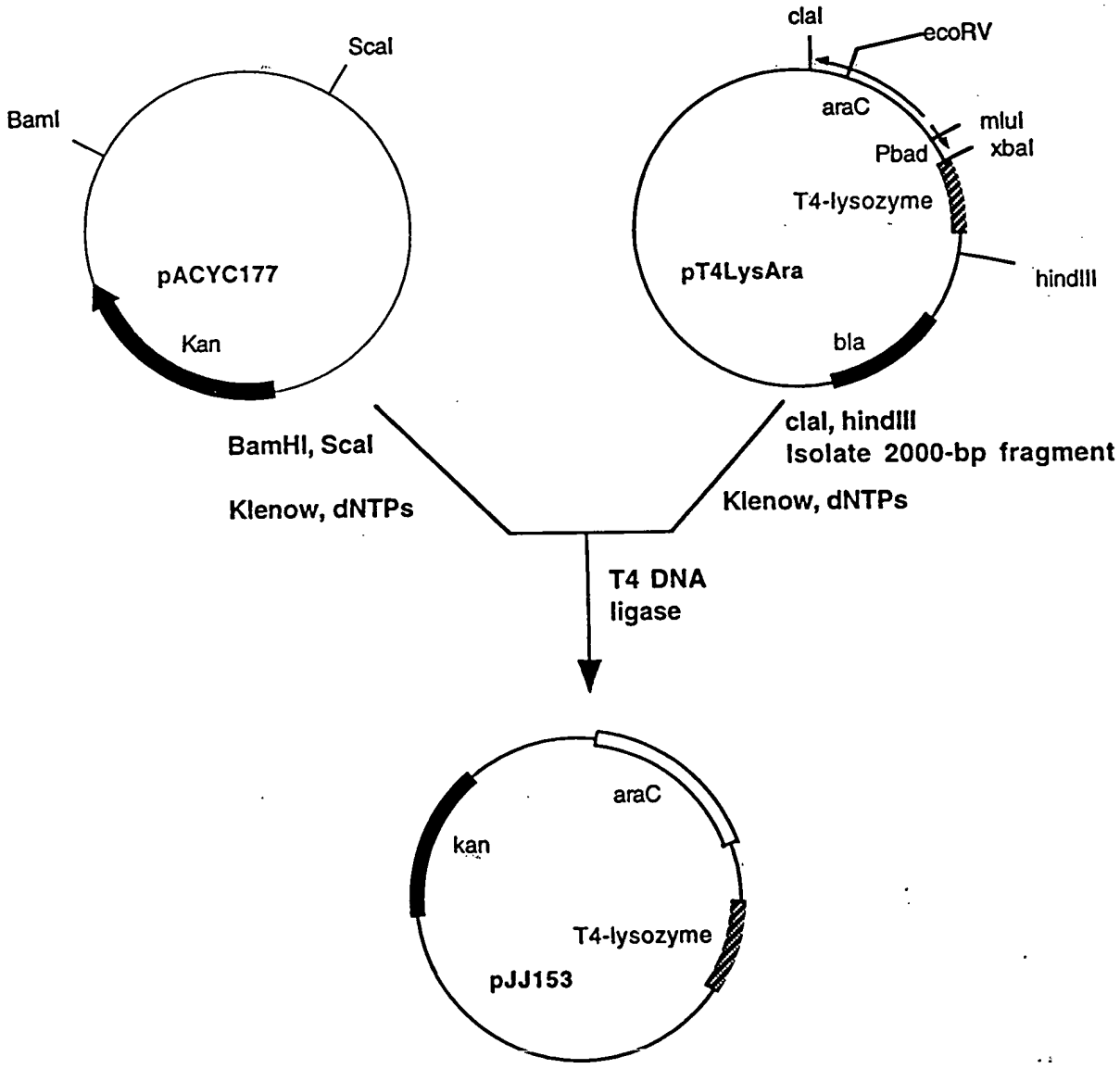


Fig. 12

Fig. 13

pJJ153 Plasmid Construction for Co-expression of ara-driven T4-Lysozyme



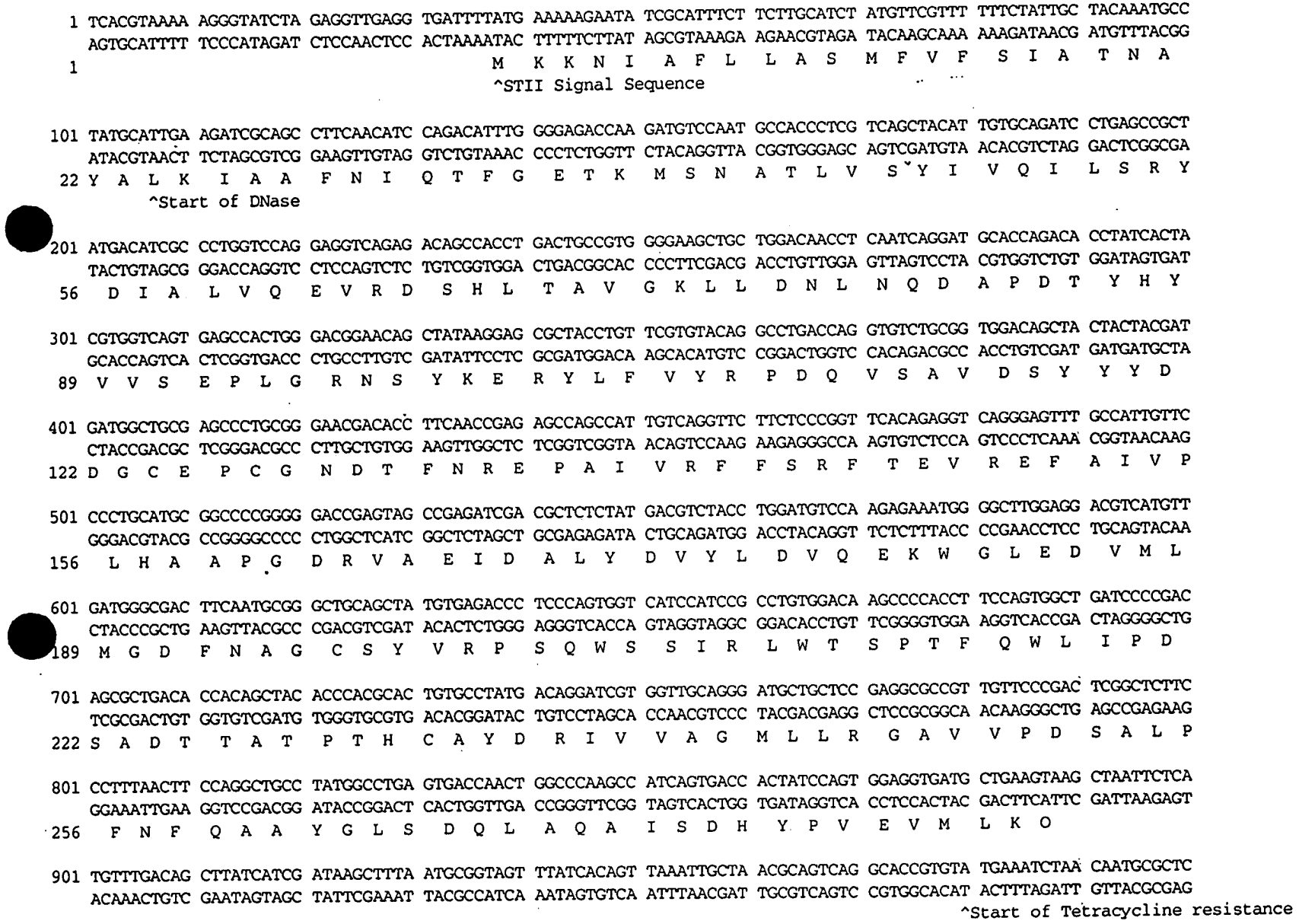

1 TCACGTAAAA AGGGTATCTA GAGGTTGAGG TGATTTTATG AAAAAGAATA TCGCATTTCT TCTTGCATCT ATGTTCGTTT TTTCTATTGC TACAAATGCC
AGTGCATTTT TCCCATAGAT CTCCAACTCC ACTAAAAATAC TTTTTCTTAT AGCGTAAAGA AGAACGTAGA TACAAGCAAA AAAGATAACG ATGTTTACGG
1 M K K N I A F L L A S M F V F S I A T N A
^STII Signal Sequence
101 TATGCATTGA AGATCGCAGC CTTCAACATC CAGACATTTG GGGAGACCAA GATGTCCAAT GCCACCCTCG TCAGCTACAT TGTGCAGATC CTGAGCCGCT
ATACGTAACT TCTAGCGTCG GAAGTTGTAG GTCTGTAAAC CCCTCTGGTT CTACAGGTTA CGGTGGGAGC AGTCGATGTA ACACGTCTAG GACTCGGCGA
22 Y A L K I A A F N I Q T F G E T K M S N A T L V S Y I V Q I L S R Y
^Start of DNase
201 ATGACATCGC CCTGGTCCAG GAGGTCAGAG ACAGCCACCT GACTGCCGTG GGGAAGCTGC TGGACAACCT CAATCAGGAT GCACCAGACA CCTATCACTA
TACTGTAGCG GGACCAGGTC CTCCAGTCTC TGTCGGTGGA CTGACGGCAC CCCTTCGACG ACCTGTTGGA GTTAGTCCTA CGTGGTCTGT GGATAGTGAT
56 D I A L V Q E V R D S H L T A V G K L L D N L N Q D A P D T Y H Y
301 CGTGGTCAGT GAGCCACTGG GACGGAACAG CTATAAGGAG CGCTACCTGT TCGTGTACAG GCCTGACCAG GTGTCTGCGG TGGACAGCTA CTACTACGAT
GCACCAGTCA CTCGGTGACC CTGCCTTGTC GATATTCCTC GCGATGGACA AGCACATGTC CGGACTGGTC CACAGACGCC ACCTGTCGAT GATGATGCTA
89 V V S E P L G R N S Y K E R Y L F V Y R P D Q V S A V D S Y Y Y D
401 GATGGCTGCG AGCCCTGCGG GAACGACACC TTCAACCGAG AGCCAGCCAT TGTCAGGTTC TTCTCCCGGT TCACAGAGGT CAGGGAGTTT GCCATTGTTC
CTACCGACGC TCGGGACGCC CTTGCTGTGG AAGTTGGCTC TCGGTCGGTA ACAGTCCAAG AAGAGGGCCA AGTGTCTCCA GTCCCTCAAA CGGTAACAAG
122 D G C E P C G N D T F N R E P A I V R F F S R F T E V R E F A I V P
501 CCCTGCATGC GGCCCCGGGG GACCGAGTAG CCGAGATCGA CGCTCTCTAT GACGTCTACC TGGATGTCCA AGAGAAATGG GGCTTGGAGG ACGTCATGTT
GGGACGTACG CCGGGGCCCC CTGGCTCATC GGCTCTAGCT GCGAGAGATA CTGCAGATGG ACCTACAGGT TCTCTTTACC CCGAACCTCC TGCAGTACAA
156 L H A A P G D R V A E I D A L Y D V Y L D V Q E K W G L E D V M L
601 GATGGGCGAC TTCAATGCGG GCTGCAGCTA TGTGAGACCC TCCCAGTGGT CATCCATCCG CCTGTGGACA AGCCCCACCT TCCAGTGGCT GATCCCCGAC
CTACCCGCTG AAGTTACGCC CGACGTCGAT ACACTCTGGG AGGGTCACCA GTAGGTAGGC GGACACCTGT TCGGGGTGGA AGGTCACCGA CTAGGGGCTG
189 M G D F N A G C S Y V R P S Q W S S I R L W T S P T F Q W L I P D
701 AGCGCTGACA CCACAGCTAC ACCCACGCAC TGTGCCTATG ACAGGATCGT GGTTGCAGGG ATGCTGCTCC GAGGCGCCGT TGTTCCCGAC TCGGCTCTTC
TCGCGACTGT GGTGTCGATG TGGGTGCGTG ACACGGATAC TGTCCTAGCA CCAACGTCCC TACGACGAGG CTCCGCGGCA ACAAGGGCTG AGCCGAGAAG
222 S A D T T A T P T H C A Y D R I V V A G M L L R G A V V P D S A L P
801 CCTTTAACTT CCAGGCTGCC TATGGCCTGA GTGACCAACT GGCCCAAGCC ATCAGTGACC ACTATCCAGT GGAGGTGATG CTGAAGTAAG CTAATTCTCA
GGAAATTGAA GGTCCGACGG ATACCGGACT CACTGGTTGA CCGGGTTCGG TAGTCACTGG TGATAGGTCA CCTCCACTAC GACTTCATTC GATTAAGAGT
256 F N F Q A A Y G L S D Q L A Q A I S D H Y P V E V M L K O
901 TGTTTGACAG CTTATCATCG ATAAGCTTTA ATGCGGTAGT TTATCACAGT TAAATTGCTA ACGCAGTCAG GCACCGTGTA TGAAATCTAA CAATGCGCTC
ACAAACTGTC GAATAGTAGC TATTCGAAAT TACGCCATCA AATAGTGTCA ATTTAACGAT TGCGTCAGTC CGTGGCACAT ACTTTAGATT GTTACGCGAG
^Start of Tetracycline resistance

FIG 17

FIG. 15

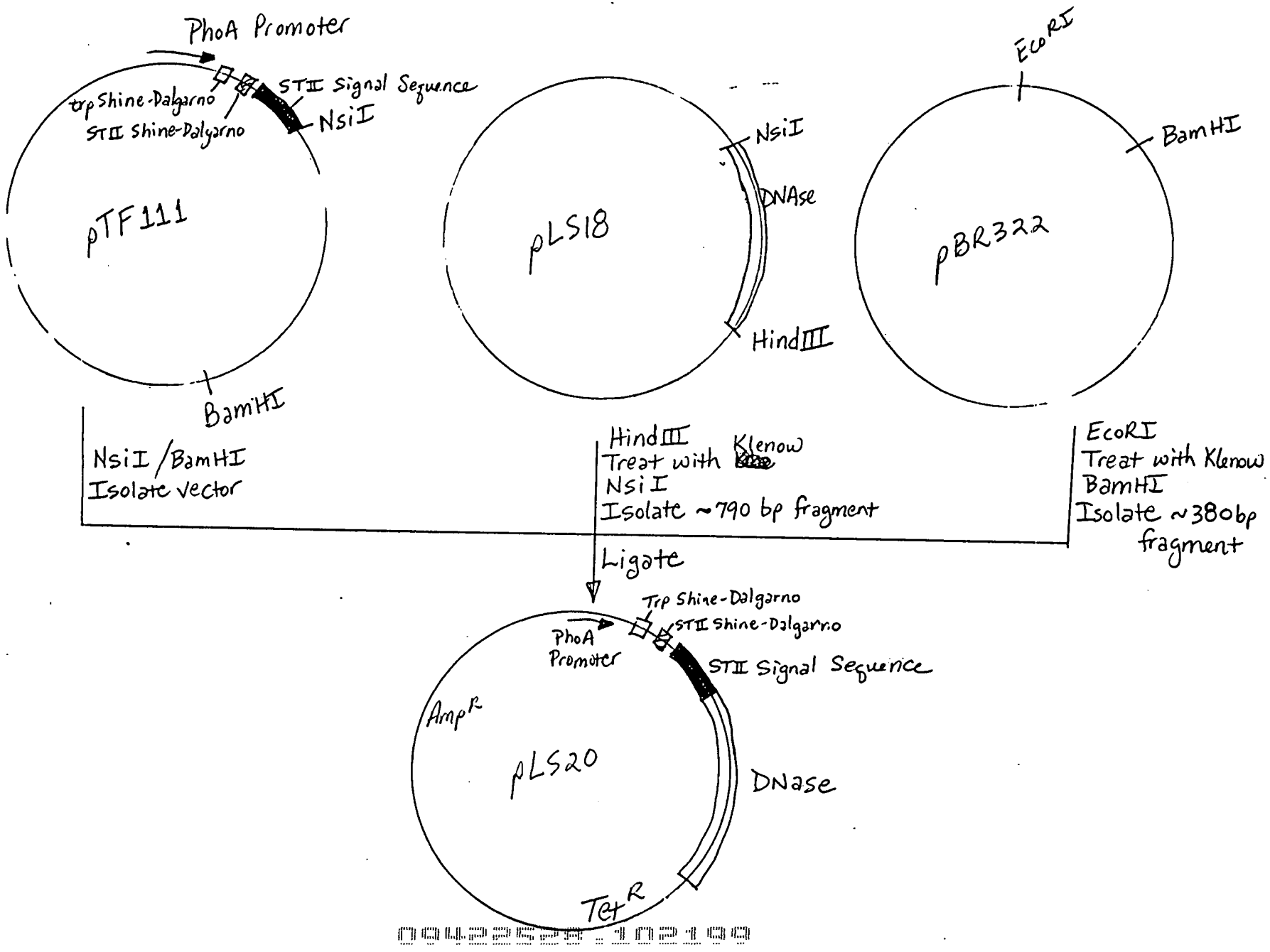

PhoA Promoter
Trp Shine-Dalgarno
STII Shine-Dalgarno
STII Signal Sequence
NsiI
pTF111
BamHI
NsiI /BamHI
Isolate vector
pLS18
NsiI
DNAse
HindIII
HindIII
Treat with Klenow
NsiI
Isolate ~790 bp fragment
pBR322
EcoRI
BamHI
EcoRI
Treat with Klenow
BamHI
Isolate ~380bp fragment
Ligate
Trp Shine-Dalgarno
STII Shine-Dalgarno
PhoA Promoter
STII Signal Sequence
Amp^R
pLS20
DNase
Tet^R

FIG. 16

RP Recovery Process Evaluation
VEGF Broth Induced for T4-Lysozyme Co-expression

Gel Analysis of RP Recovered:

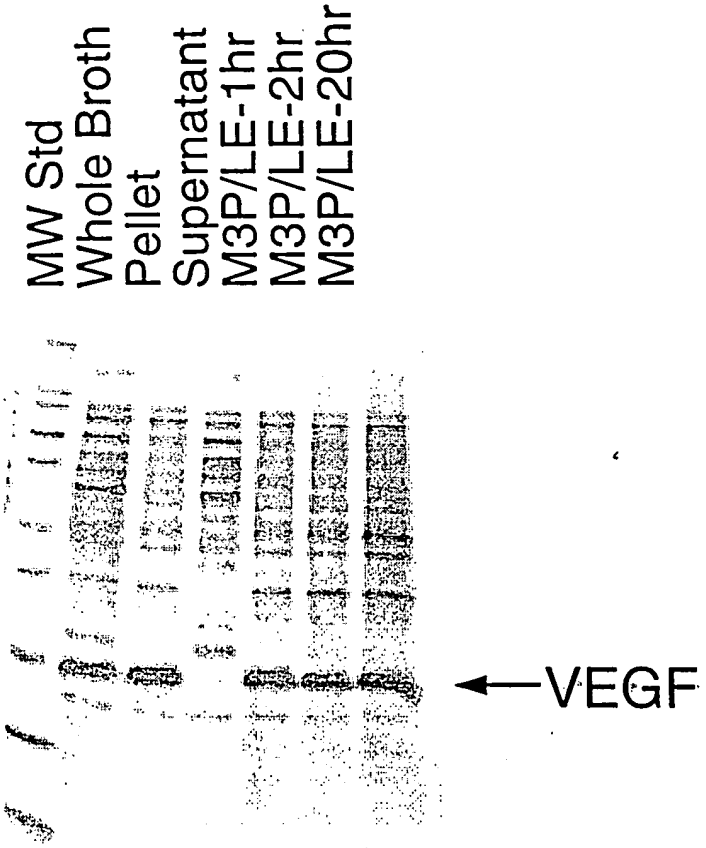


FIG. 17

RP Recovery Process Evaluation
DNase Broth Induced for T4-Lysozyme Co-expression

Gel Analysis of RP Recovered: